SUPPLEMENTARY MATERIALS

Crystal structure and functional analysis of MiD49, a receptor for the mitochondrial fission protein Drp1

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Supplementary materials: Table S1, Figure S1, Figure S2 and figure legends

Table S1. Data Collection and Refinement Statistics

Data	Molecular Replacement
Space group	C 2 2 21
Unit cell (Å, °)	100.7, 153.4, 109.0, 90, 90, 90
Number of molecules in ASU	2
Resolution (Å)	36.98 - 2.40 (2.48 - 2.40)[a]
R_{merge} (%)[b]	5.1 (69.4)
Completeness (%)	98.92 (99.51)
Mean I/σ	21.5 (2.1)
Number of measured reflections	142983 (20,782)
Number of unique reflections	32951 (4,777)
Redundancy	4.3 (4.4)
R_{work} (%)[c]	20.9
R_{free} (%)	24.4
Average B-factor (Å^2)	50.45
Rmsd from ideal values	
Bonds (Å)	0.010
Angle (°)	1.36
Ramachandran statistics (%)	
Favored	97.71
Allowed	1.83
Outliers	0.46

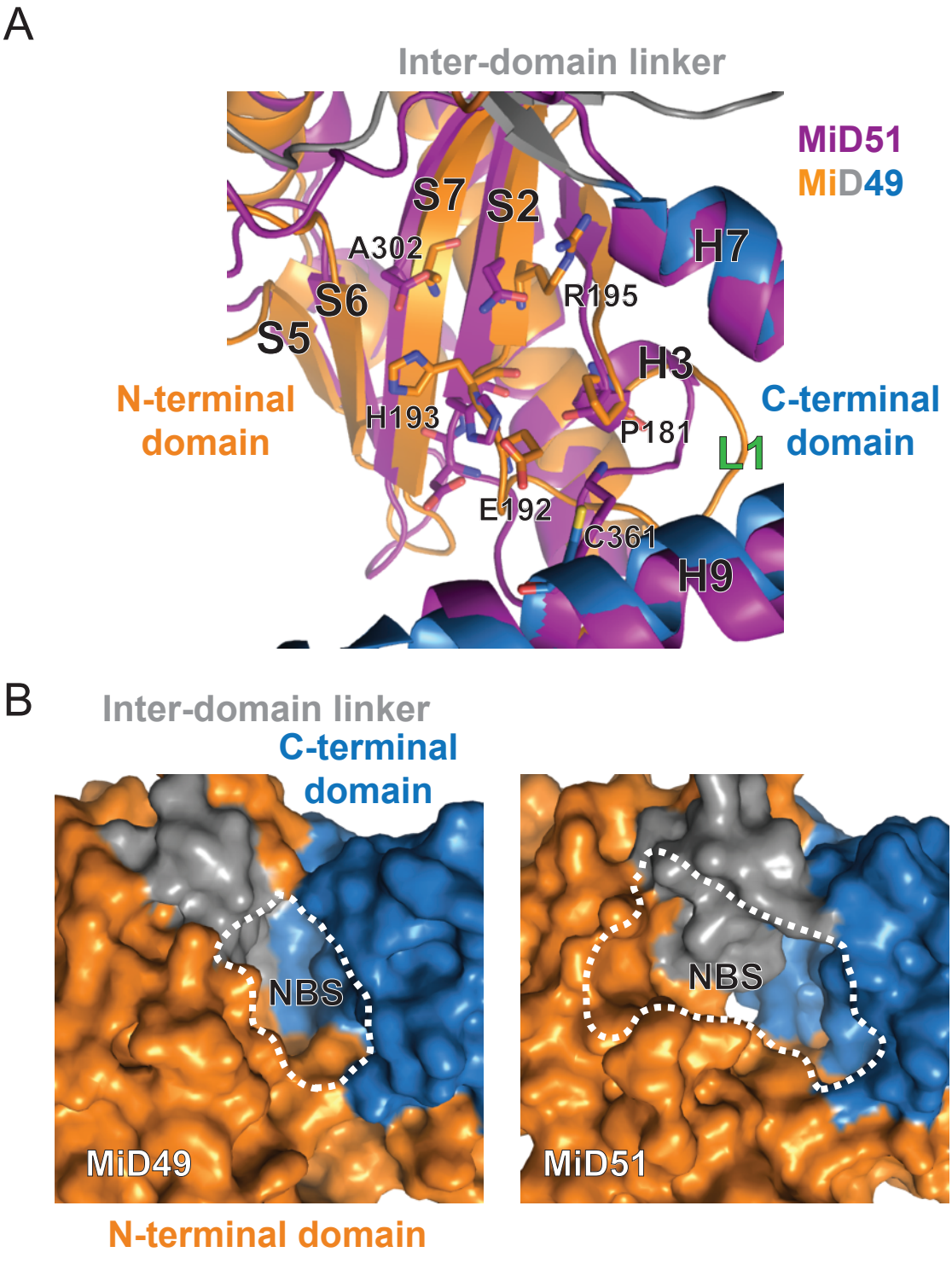
[a] Values in parentheses are for the highest resolution shell

[b] $R_{merge} = \sum\sum_i |I_i - \langle I \rangle| / \sum \langle I \rangle$, where $\langle I \rangle$ is the mean intensity of N reflections with intensities I_i and common indices *h, k,* and *l*.

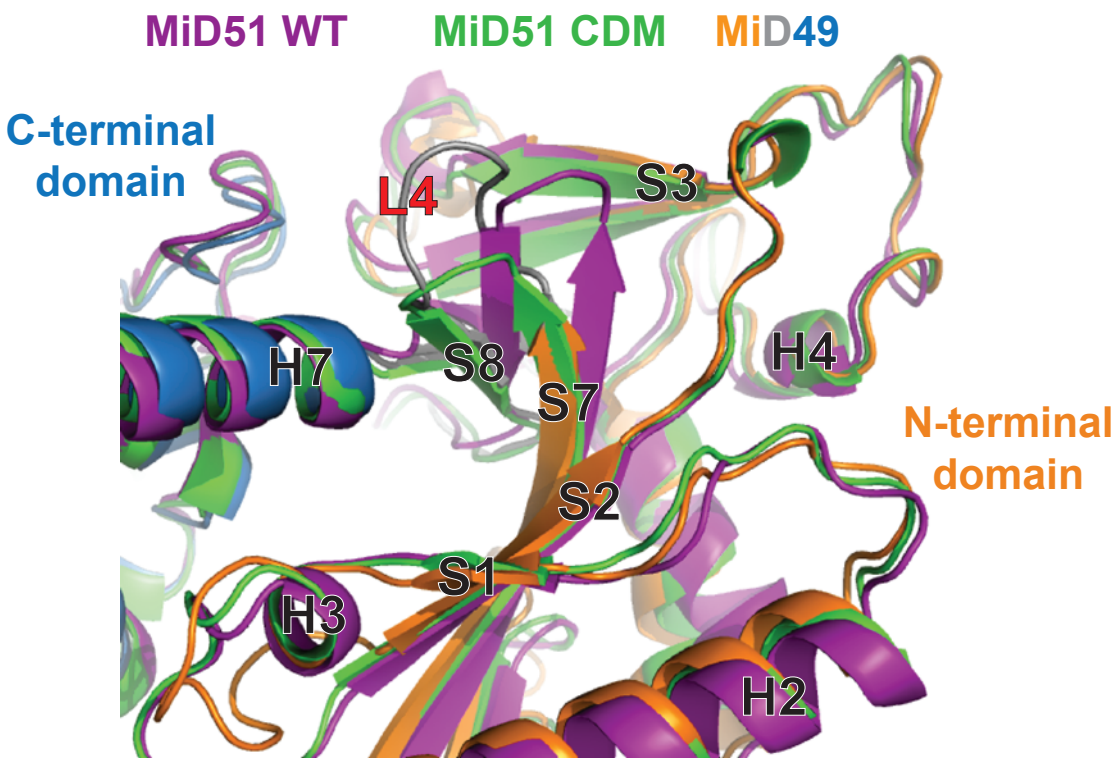
[c] $R_{work} = \sum_{hkl} ||F_{obs}| - k|F_{cal}|| / \sum_{hkl} |F_{obs}|$, where F_{obs} and F_{cal} are the observed and calculated structure factors, respectively.

Figure S1. Structural differences in the nucleotide binding sites of MiD49 and MiD51. (A) Differences in nucleotide binding residues and the central β-sheet region. Also, loop 1 (green label) is significantly rearranged in MiD49. MiD49 residues analogous to the nucleotide binding residues of MiD51 are labeled in black text. Of the key nucleotide binding residues in MiD51, only the histidine (H193) is conserved in MiD49. Secondary structure labeling and domain coloring for MiD49 are as in Fig. 2. Purple, MiD51. (B) Surface representation of the putative nucleotide binding sites (NBS) of MiD49 (left) and MiD51 (right). The binding site opening is outlined by a dotted white line. Domains are colored as in Fig. 2.

Figure S2. Structural differences outside the nucleotide binding sites of MiD49 and MiD51. (A) Loop 4 (red label) shifts downward in MiD49 in a similar manner to the MiD51 dimer mutant (CDM; compound dimer mutant). Secondary structure labeling and domain coloring for MiD49 are as in Fig. 2. In this image, the molecule is viewed from the back, similar to the right view in Fig. 2B. However, the molecule is rotated clockwise such that the membrane proximal direction is towards the left. Purple, MiD51 WT; green, MiD51 CDM. (B) Sequence alignment of dimer interface region. Red, MiD51 residues that were previously found to be critical for dimer formation (14). Sequence similarity symbols: asterisk, fully conserved; colon, highly conserved; period, weakly conserved.


A
Inter-domain linker
MiD51
MiD49
S7
S2
A302
H7
S6
R195
S5
H3
N-terminal domain
H193
P181
C-terminal domain
L1
E192
C361
H9
B
Inter-domain linker
C-terminal domain
NBS
MiD49
NBS
MiD51
N-terminal domain

A



B

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MiD49 155 DIALELQAYLRSKFPELPFGALVPGGP 181
MiD51 163 DICAELRSFLRAKLPDMPLRDMYLSGS 189
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